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# Relative effectiveness of calcium and magnesium in the alleviation of rhizotoxicity in wheat induced by copper, zinc, aluminum, sodium, and low pH

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## Abstract

Root elongation in short-term experiments with wheat (*Triticum aestivum* L.) seedlings demonstrated that the following ions were rhizotoxic in the order $Cu^{2+} > Al^{3+} \gg H^{+} > Zn^{2+} \gg Na^{+}$. Additions of $Ca^{2+}$ and $Mg^{2+}$ alleviated the toxicity, but the relative ameliorative effectiveness of $Ca^{2+}$ and $Mg^{2+}$ depended upon the toxicant. The effectiveness of $Mg^{2+}$ relative to $Ca^{2+}$ was 0.098 for $Na^{+}$, 0.37 for $H^{+}$, 1.0 for $Al^{3+}$, 2.1 for $Cu^{2+}$, and 170 for $Zn^{2+}$. The mechanisms of inhibition are mainly unknown, but the mechanisms of alleviation are better understood. *Mechanism I* entails ameliorant-induced reduction of the negativity of the plasma membrane (PM) surface electrical potential ($\psi_0$). The consequence is a reduced activity of the toxicant at the PM surface because of reduced electrostatic attraction. $Ca^{2+}$ and $Mg^{2+}$ are equally effective agents of Mechanism I alleviation but are less effective than $H^{+}$ and more effective than $Na^{+}$ for reasons described by electrostatic principles. *Mechanism II* alleviation is specific for $Ca^{2+}$ and entails the restoration of $Ca^{2+}$ at the PM surface if surface $Ca^{2+}$ has been reduced by the toxicant to growth-limiting levels. This occurs more commonly in $Na^{+}$ and $H^{+}$ toxicities than in the others, though in no case is it the principal mechanism of alleviation. *Mechanism III* alleviation is the collective ameliorative effect of an ion beyond Mechanisms I and II. Differences between $Ca^{2+}$ and $Mg^{2+}$ in ameliorative effectiveness are mainly attributable to Mechanism III which, in the case of $Zn^{2+}$, may entail an internal detoxification and, in the case of $Na^{+}$, may entail the blockade of a $Na^{+}$ uptake channel. This study demonstrates that appropriate nonlinear equations incorporating cell-surface ion activities enable the dissection of multiple toxic and ameliorative effects of the ions.

*Abbreviations:* PM – plasma membrane; *RRL* – relative root length expressed as percent; $\{I^Z\}$ – activity of ion $I^Z$ in the bulk phase of the rooting medium; $\{I^Z\}_0$ – activity of ion $I^Z$ at the outer surface of the PM; {T} – activity of toxicant T in the bulk phase of the rooting medium; $\{T\}_0$ – activity of toxicant T at the outer surface of the PM; $\psi_0$ – electrical potential at the outer surface of the PM.

## Introduction

Many mineral elements, including essential nutrient elements, may be phytotoxic at excessive concentrations (Marschner, 1995). Recent studies, together with the present study, allow a comparison of the rhizotoxic effects of $Al^{3+}$, $Cu^{2+}$, $Zn^{2+}$, $Na^{+}$, and $H^{+}$ in wheat seedlings (*Triticum aestivum* L.) (Kinraide, 1998; Parker et al., 1998; Pedler et al., 2004). These studies also allow a comparison of the ameliorative effects of $Ca^{2+}$ and $Mg^{2+}$. The mechanisms of toxicity are incompletely understood, but the alleviation

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of toxicity by $Ca^{2+}$, $Mg^{2+}$, and other cations is better understood. For $Ca^{2+}$, three mechanism of alleviation have been identified (Kinraide, 1998).

*Mechanism I* is the electrostatic displacement of cationic toxicants from the plasma membrane (PM) surface. Addition of $Ca^{2+}$ salts to the rooting medium causes a reduction in the negative potential at the outer surface of the PM because of ionic screening and binding, thereby reducing the electrostatic attraction of cationic toxicants. Because of their equal charge and strength of binding to the PM, $Ca^{2+}$ and $Mg^{2+}$ have equal effectiveness as Mechanism I ameliorants. $Al^{3+}$ and $H^+$ have even higher Mechanism I effectiveness (Grauer and Horst, 1990; Kinraide, 2003) even though both ions are also intoxicating. $Na^+$ and $K^+$ also alleviate toxicity by Mechanism I, but much more weakly than $Ca^{2+}$ and $Mg^{2+}$. These relative effects can be understood in term of ion binding (see *Computation of ion activities at the PM surface below*).

*Mechanism II* is the restoration of $Ca^{2+}$ at the PM surface. Extracellular $Ca^{2+}$ is essential for root elongation even in the absence of toxicants. If a toxicant has sufficiently displaced $Ca^{2+}$ from the PM surface (by toxicant-induced reduction of surface negativity, or by other means), then the addition of $Ca^{2+}$ will engage Mechanism II. $Mg^{2+}$, of course, has no Mechanism II effectiveness; in fact, it may induce $Ca^{2+}$ insufficiency. Induced $Ca^{2+}$ insufficiency is a component, though not usually the major component, of toxicity induced by low pH or high salinity (Kinraide, 1998, 1999).

*Mechanism III* is the residual alleviation beyond Mechanisms I and II. It is a heterogeneous suite of mechanisms that may entail interactions between $Ca^{2+}$ and the toxicant at the PM surface. For example, Mechanism III alleviation of $Na^+$ almost certainly includes the blockade of ion channels that admit $Na^+$ to the cell interior (Tyerman, 1997). $Mg^{2+}$ also has Mechanism III activity (see below). The relative Mechanism III effectiveness of $Ca^{2+}$ and $Mg^{2+}$ depends upon the ions whose toxicities they alleviate. Furthermore, the relative effectiveness depends upon genotype. In soybean, $Mg^{2+}$ is much more effective than $Ca^{2+}$ in the alleviation of $Al^{3+}$ toxicity (Silva et al., 2001a, 2001b). In wheat, $Mg^{2+}$ has no special effectiveness in the alleviation of $Al^{3+}$ toxicity (Kinraide, 1998), but $Mg^{2+}$ is much more effective than $Ca^{2+}$ in the alleviation of $Zn^{2+}$ toxicity (Pedler et al., 2004).

This investigation presents a quantitative assessment of the interactions among toxic and ameliorative ions, especially $Ca^{2+}$ and $Mg^{2+}$. Our interest was stimulated by recent discoveries of a remarkable alleviation of some toxicities by $Mg^{2+}$ at low concentrations.

## Materials and methods

### *Growth experiments*

Data for root elongation in response to toxicants and ameliorants were compiled for analysis from previous publications (Kinraide, 1998, 1999; Parker et al., 1998), from the accompanying article (Pedler et al., 2004), and from some of our unpublished studies. Root elongation was assessed by the cultivation of 2-d-old wheat seedlings in aerated solutions at 25 °C in the dark for an additional 2 d according to methods described previously. Additional details of the culture conditions, including solutes and wheat genotypes are presented with the results.

### *Computation of ion activities at the PM surface*

The activity of ion $I^Z$ at the surface of the PM ($\{I^Z\}_0$) was computed from the activity of $I^Z$ in the bulk-phase medium ($\{I^Z\}$) according to the Nernst equation ($\{I^Z\}_0 = \{I^Z\} \exp[-Z_i F \psi_0/(RT)]$). $\psi_0$ is the electrical potential at the PM surface and was computed by a Gouy-Chapman-Stern model (Yermiyahu et al., 1997; Kinraide et al., 1998). F, R, and T are the Faraday constant, the gas constant, and the temperature.

Binding constants are among the parameters required for the Gouy-Chapman-Stern model. We set $K_{R,Zn} = K_{R,Cu} = K_{R,Mg} = K_{R,Ca} = 30\ M^{-1}$, which refers to the binding of the ions to a negative membrane site designated $R^-$. (For comparison, $K_{R,H} = 21,500$, $K_{R,Al} = 20,000$, $K_{R,La} = 2200$, $K_{R,Na} = K_{R,K} = 1\ M^{-1}$ [Yermiyahu et al., 1997].) There is experimental justification for the value for $Ca^{2+}$ and $Mg^{2+}$ (Kinraide, 2001), but not for $Zn^{2+}$ and $Cu^{2+}$. Figure 1 presents plots of surface activities of these ions computed on the basis of $K_{R,Zn} = 300\ M^{-1}$ vs. $K_{R,Zn} = 30\ M^{-1}$ or $K_{R,Cu} = 3000\ M^{-1}$ vs. $K_{R,Cu} = 30\ M^{-1}$. Clearly, differences in binding strength for $Cu^{2+}$ and $Zn^{2+}$ make little difference when their activities are low relative to the activities of other ions such as $Ca^{2+}$, $Mg^{2+}$, and $H^+$, which in these experiments were the principal controllers of $\Psi_0$. These results demonstrate that in our experiments the computation of $\{Zn^{2+}\}_0$ and $\{Cu^{2+}\}_0$ was insensitive

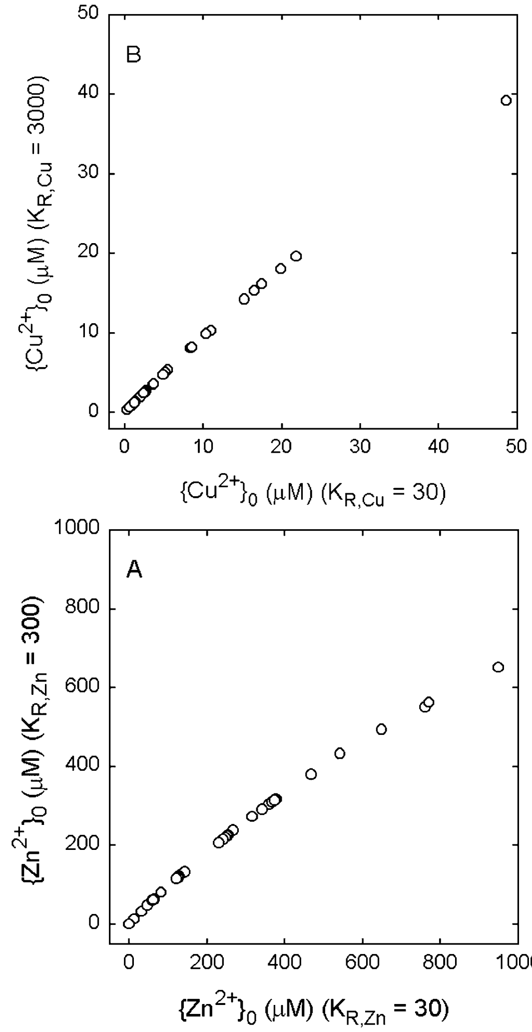


*Figure 1.* Comparison of computed PM surface activity of $Zn^{2+}$ and $Cu^{2+}$ assuming different binding constants. A. $\{Zn^{2+}\}_0$, assuming $K_{R,Zn} = 300$, vs. $\{Zn^{2+}\}_0$, assuming $K_{R,Zn} = 30$. B. $\{Cu^{2+}\}_0$, assuming $K_{R,Cu} = 3000$, vs. $\{Cu^{2+}\}_0$, assuming $K_{R,Cu} = 30$.

to the binding of these ions, not that the assumed value of 30 $M^{-1}$ is correct.

*Analysis of root elongation*

$RL$ is the mean length of roots from a solution in which the two longest roots from each of five seedlings were measured. Relative root length ($RRL$) is defined as $100(RL_T - RL_S)/(RL_C - RL_S)$ where $RL_T$ is the $RL$ in the presence of toxicant, $RL_C$ is the $RL$ in the corresponding $Ca^{2+}$-sufficient, toxicant-free control, and $RL_S$ is the $RL$ in the presence of toxicant sufficient to minimize growth.

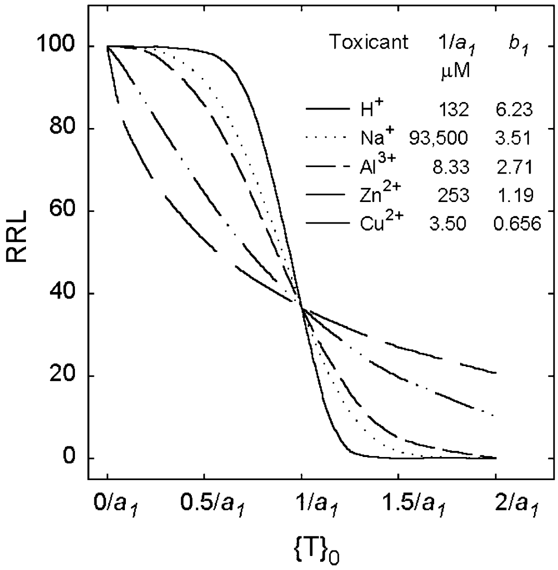


*Figure 2.* Illustration of the Equation 1 ($RRL = 100/\exp[(a_1\{T\}_0)^{b1}]$). The values for the coefficients $a_1$ and $b_1$ were taken from Table 2.

Growth may be plotted against measures of toxicant intensity, such as the PM surface activity of toxicant T ($\{T\}_0$). Expressing T as $\{T\}_0$ instead of $\{T\}$ takes Mechanism I into account. The resulting curves are often downwardly sigmoidal and have been expressed by a Weibull equation (Kinraide, 2003; Pedler et al., 2004). If growth is limited only by $\{T\}_0$, then

$$RRL = 100/\exp\left[(a_1\{T\}_0)^{b1}\right], \tag{1}$$

where $a_1$ and $b_1$ are coefficients that can be evaluated by regression analysis. $a_1$ increases with the strength of the toxicant, and $b_1$ confers sigmoidality when its value is greater than 1. Figure 2 illustrates the properties of Equation 1. When $\{T\}_0 = 1/a_1$ then $(a_1\{T\}_0)^{b1} = 1$, irrespective of the value of $b_1$, and RRL = 36.8. The coefficient $b_1$ controls the steepness of the curve in the region of RRL = 36.8

If $Ca^{2+}$ insufficiency limits growth (because of toxicant displacement of $Ca^{2+}$ from the PM surface, or for any other reason), then the addition of $Ca^{2+}$ may enhance growth, and plots of growth vs. $\{Ca^{2+}\}_0$ may be upwardly sigmoidal (complementary to the curves in Figure 2). If growth is limited only by $\{Ca^{2+}\}_0$ insufficiency, then

$$RRL = 100 - 100/\exp[(a_2\{Ca^{2+}\}_0)^{b2}]. \tag{2}$$

This equation expresses Mechanism II alleviation.

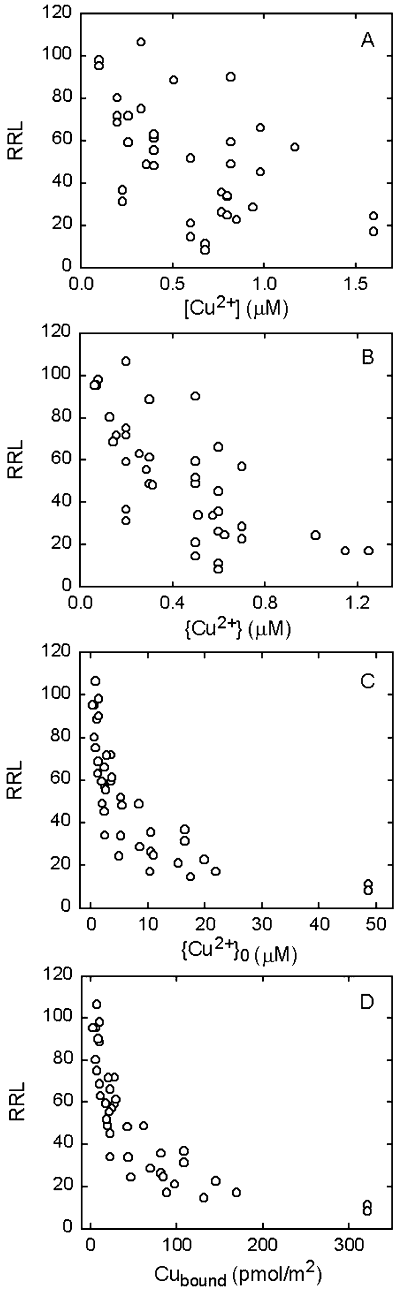


*Figure 3.* Root growth responses of Yecora Rojo wheat seedlings to $Cu^{2+}$. Data are compiled from Figures 1 through 5 from Parker et al. (1998). A. Responses to $Cu^{2+}$ concentration in the rooting medium. B. Responses to $Cu^{2+}$ activity in the rooting medium. C. Responses to the computed $Cu^{2+}$ activity at the PM surface. D. Responses to the computed $Cu^{2+}$ bound to the PM surface. $Cu_{bound}$, but not $\{Cu^{2+}\}_0$, was sensitive to changes in the binding affinity of $Cu^{2+}$.

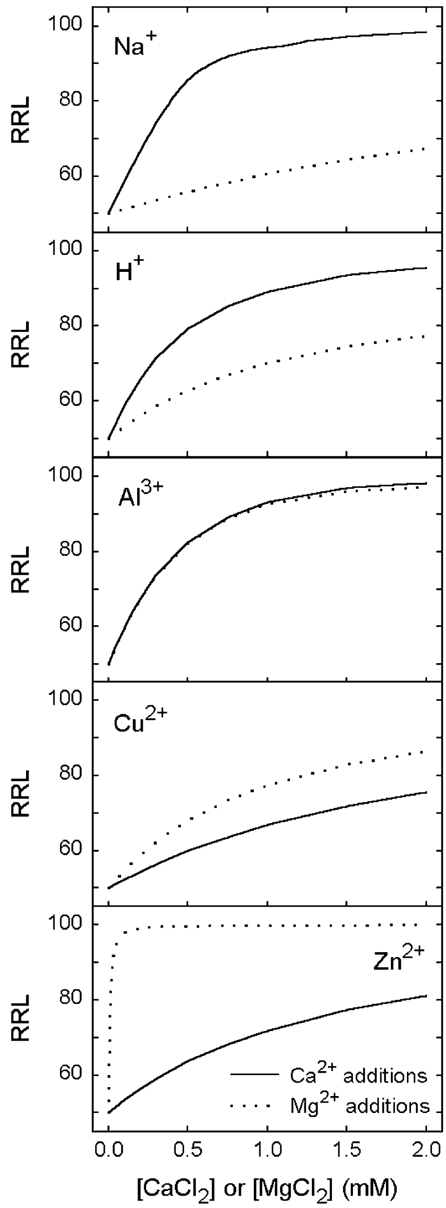


*Figure 4.* Computed growth responses of mineral-intoxicated roots to supplemental $CaCl_2$ or $MgCl_2$. For $Na^+$, $H^+$, and $Al^{3+}$, growth was computed according to the equation and parameters presented in Table 2 or in the text for $Cu^{2+}$ and $Zn^{2+}$. The basal medium for each toxicant (selected so that $RRL = 50$) was 129.1 mM NaCl at pH 5 for $Na^+$; pH 4.173 for $H^+$; 0.766 $\mu$M $AlCl_3$ at pH 5 for $Al^{3+}$; 0.2385 $\mu$M $CuCl_2$ at pH 6 for $Cu^{2+}$; and 12.44 $\mu$M $ZnCl_2$ at pH 6 for $Zn^{2+}$. In addition, all solutions contained a basal concentration of 0.5 mM $CaCl_2$.

The joint effect of a toxicant and $Ca^{2+}$ limitation can be expressed in a multiplicative model

$$RRL = 100 RRL_T \cdot RRL_{Ca} = 100/\exp[(a_1\{T\}_0)^{b1}] \\ (1 - 1/\exp[(a_2\{Ca^{2+}\}_0)^{b2}]) \tag{3}$$

To express $RRL$ on a scale of 0 to 100, $RRL_T$ and $RRL_{Ca}$ must be expressed on a scale of 0 to 1. Equations incorporating the term $(1 - 1/\exp[(a_2\{Ca^{2+}\}_0)^{b2}])$ can be evaluated only for experiments where $\{Ca^{2+}\}_0$ was low enough to limit root elongation in some of the treatments, otherwise the term is consistently equal to 1 and Mechanism II is not engaged.

It is possible that one or more ameliorants (often $Ca^{2+}$) interacts with the toxicant at the PM (by channel blockade, for example). In that case Equation 3 is inadequate. A possible way to express the interactions would be to incorporate a dependence upon the ameliorants into the coefficient for $\{T\}_0$. Thus $a_1$ could be written

$$a_1 = a_{11}/\left(1 + a_{12}\{Ca^{2+}\}_0\right) \tag{4}$$

so that $a_1$ decreases as $\{Ca^{2+}\}_0$ increases. Incorporation of $\left(1 + a_{12}\{Ca^{2+}\}_0\right)$ is a quantitative expression of Mechanism III effects. Some solutions will contain more than one ameliorant. The following equation expresses the Mechanism III effectiveness of both $Ca^{2+}$ and $Mg^{2+}$ alleviation of $Zn^{2+}$ toxicity, and the coefficients $a_{12}$ and $a_{13}$ quantify their ameliorative strength.

$$RRL = 100/\exp\left[(a_{11}/(1 + a_{12}\{Ca^{2+}\}_0 + a_{13}\{Mg^{2+}\}_0)\{Zn^{2+}\}_0)^{b1}\right] \\ \left(1 - 1/\exp[(a_2\{Ca^{2+}\}_0)^{b2}]\right). \tag{5}$$

## Results

### $Cu^{2+}$ *toxicity*

Parker et al. (1998) presented results from experiments assessing wheat root (cv. Yecora Rojo) elongation in response to $CuCl_2$, $CaCl_2$, $MgCl_2$, and pH. The solutions contained 0.25 mM MES adjusted to pH 6 with NaOH. In a preliminary evaluation of ionic effects we reevaluated their results first with the following equation in which bulk-phase activities were used rather than PM-surface activities. This equation allows a comparison of overall ameliorative effectiveness but does not dissect the modes of amelioration.

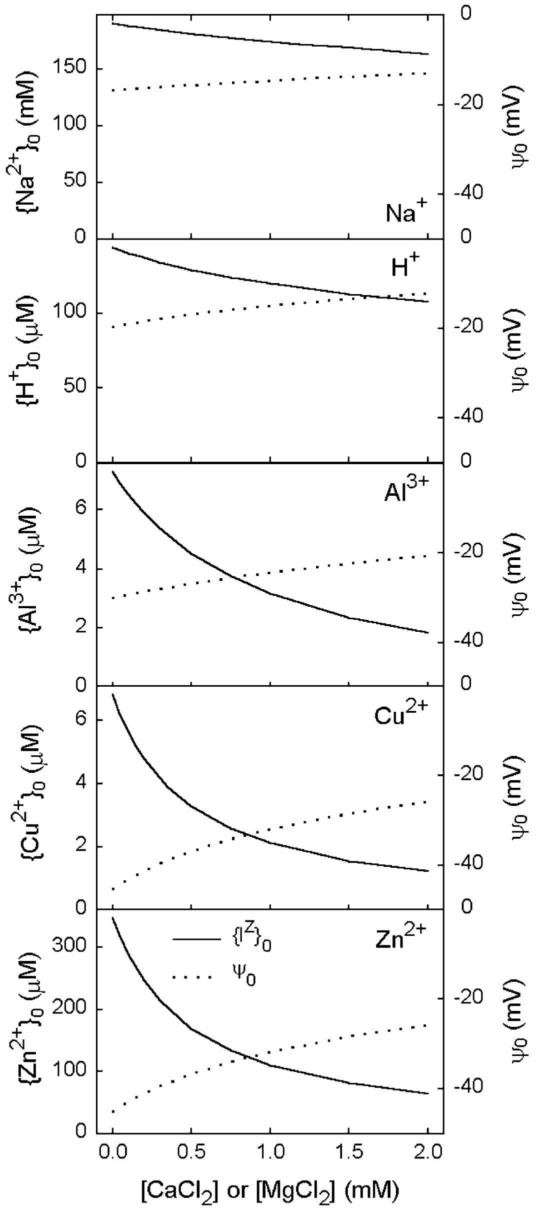


*Figure 5.* PM electrical potential and surface activities of mineral toxicants in response to supplemental $CaCl_2$ or $MgCl_2$. The conditions are those presented in Figure 4 so $RRL = 50$ at zero supplemental $CaCl_2$ or $MgCl_2$.

$$RRL = 100/\exp\left[(a_{11}/(1 + a_{12}\{Ca^{2+}\} + a_{13}\{Mg^{2+}\} + a_{14}\{H^+\})\{Cu^{2+}\})^{b1}\right]. \tag{6}$$

Regression analysis yielded these values for the coefficients: $a_{11} = 7600$, $a_{12} = 0.694$, $a_{13} = 1.86$, $a_{14} = 90.3$, $b_1 = 1.04$. $R^2 = 0.934$; $n = 39$. Thus $H^+$ alleviates $Cu^{2+}$ toxicity about 70 times more effectively than $Ca^{2+}$ or $Mg^{2+}$ even while $H^+$ was exerting its own toxicity. $Mg^{2+}$ may be somewhat more effective than $Ca^{2+}$, as noted by Parker et al. (1998), but the 95% confidence intervals for $a_{12}$ and $a_{13}$ overlap.

To discern the modes of alleviation the growth data were analyzed by Equation 5. Elimination of nonsignificant terms reduced the equation to $RRL = 100/\exp\left[(a_{11}\{Cu^{2+}\}_0)^{b1}\right]$ for which the coefficients were $a_{11} = 286$ and $b_1 = 0.656$. $R^2 = 0.805$; $n = 39$. Figure 3 presents plots of $RRL$ as a function of $Cu^{2+}$ in several different phases. Clearly, growth is better correlated with surface activities of $Cu^{2+}$ than with bulk-phase activities of $Cu^{2+}$ alone. Thus Mechanism I may be the only, or at least the principal, mechanism of alleviation, and the coefficients of Equation 6 quantify the relative effectiveness of $Ca^{2+}$, $Mg^{2+}$, and $H^+$ in Mechanism I alleviation.

### $Zn^{2+}$ *toxicity*

The accompanying article (Pedler et al., 2004) demonstrates the high ameliorative effectiveness of $Mg^{2+}$, relative to $Ca^{2+}$, for $Zn^{2+}$-intoxicated roots. The experiments analyzed here used the wheat cultivar Yecora Rojo and were otherwise similar to the experiments described for $Cu^{2+}$ toxicity. Additional studies by us confirm that cv. Scout 66 and cv. Yecora Rojo respond to $Cu^{2+}$, $Zn^{2+}$, $Ca^{2+}$, and $Mg^{2+}$ similarly. Table 1 presents results from one of nine growth experiments assessing root elongation in response to $ZnCl_2$, $CaCl_2$, and $MgCl_2$. Certainly $Mg^{2+}$ alleviated the inhibition much more strongly than $Ca^{2+}$. When all measurements from the nine experiments were pooled, regression analysis in terms of bulk-phase activities was possible, and the results are presented in Table 1. Values for $a_{12}$ and $a_{13}$ quantify the relative effectiveness of $Mg^{2+}$ and $Ca^{2+}$.

The data were analyzed next by Equation 5 which incorporates PM-surface activities. As in the case of $Cu^{2+}$ toxicity, $\left(1 - 1/\exp[(a_2\{Ca^{2+}\}_0)^{b2}]\right)$ was excluded from the equation. The coefficient $a_{12}$ was not statistically different from zero, meaning that all $Ca^{2+}$ effects have been accounted for by Mechanism I. The coefficient for $\{Mg^{2+}\}_0$ was significant, meaning Mechanism III activity was engaged by $Mg^{2+}$. The evaluated equation is this: $RRL = 100/\exp\left[(3.95/(1 + 10.7\{Mg^{2+}\}_0)\{Zn^{2+}\}_0)^{1.19}\right]$. $R^2 = 0.938$; $n = 66$.

### $Al^{3+}$, $Na^+$, *and* $H^+$ *toxicities*

The growth responses of wheat seedlings (cv. Scout 66) to $Al^{3+}$, $Na^+$, and $H^+$ have been considered in some detail (Kinraide, 1998). Each of these toxicities was relieved by $Ca^{2+}$ and $Mg^{2+}$ (Table 2). Computed growth responses of mineral-intoxicated roots to supplemental $CaCl_2$ or $MgCl_2$ is presented in Figure 4. For this figure the addition of $CaCl_2$ did not engage Mechanism II because of the 0.5 mM base-level $CaCl_2$ which ensured that growth is not limited directly by low $\{Ca^{2+}\}_0$. For $Al^{3+}$ there was no Mechanism III alleviation; for $Na^+$ Mechanism III was engaged by $Ca^{2+}$ and to a lesser extent by $Mg^{2+}$; for $H^+$ Mechanism III was engaged by $Ca^{2+}$ but not $Mg^{2+}$. Coefficients $a_{12}$ and $a_{13}$ in Table 2 quantify these effects.

## Discussion

It is clear that the following ions were rhizotoxic in the order $Cu^{2+} > Al^{3+} \gg H^+ > Zn^{2+} \gg Na^+$. That can be determined by inspection of coefficient $a_{11}$ in Table 2. However, the coefficient $a_1$ (or $a_{11}$) describes only partially the dose response curves for the toxicants. High values for $b_1$ signify strong responses to toxicants at $\{T\}_0 \approx 1/a1$ (Figure 2). The plotted curves are reminiscent of cooperativity entailing multiple receptor sites in enzymatic reactions, cell signaling, and other functions (Alberts et al., 1989). Such functions can act as switches, and in the case of $H^+$ toxicity, growth is switched from on to off as the PM-surface pH declines only 0.2 units from 4.0 to 3.8. However, we are not in a position to interpret the significance of the shapes of the dose response curves.

Large differences in the ameliorative effectiveness of $Ca^{2+}$ and $Mg^{2+}$ are apparent. The initial slopes of the curves in Figure 4 for $RRL$ vs. $[CaCl_2]$ and $[MgCl_2]$ may be used to measure the effectiveness of $Mg^{2+}$ relative to $Ca^{2+}$. The computed values for the ratios of these initial slopes are 0.098 for $Na^+$, 0.37 for $H^+$, 1.0 for $Al^{3+}$, 2.1 for $Cu^{2+}$, and 170 for $Zn^{2+}$. The differences between $Ca^{2+}$ and $Mg^{2+}$ cannot be attributed to Mechanism I, nor can they be attributed to Mechanism II in Figure 4 because of the basal 0.5 mM $CaCl_2$. Consequently, the differences can be attributed to Mechanism III. For $Na^+$ toxicity there is a good case that Mechanism III entails the blockade by $Ca^{2+}$of PM ion channels that admit $Na^+$ into the cells (Tyerman, 1997). Details of the Mechanism III activity of $Mg^{2+}$ are not known, but it may entail an internal detoxification of $Zn^{2+}$ rather than an inhibition of uptake (see the discussion in the accompanying article by Pedler et al., 2004). Surely the discovery that

*Table 1.* Data from one of nine experiments assessing the effectiveness of $Ca^{2+}$ and $Mg^{2+}$ in the alleviation of $Zn^{2+}$ rhizotoxicity. The medium was composed of $ZnCl_2$, $CaCl_2$, and $MgCl_2$, as noted, and 0.25 mM MES adjusted to pH 6. Relative root length ($RRL$) was computed according to the equation $RRL = 100/\exp\left[(a_{11}/(1+a_{12}\{Ca^{2+}\}+a_{13}\{Mg^{2+}\})\{Zn^{2+}\})^{b1}\right]$, where $a_{11} = 111$, $a_{12} = 1.30$, $a_{13} = 286$, $b_1 = 1.28$. $R^2 = 0.968$; $n = 66$. Concentrations and activities are expressed in mM

| $[ZnCl_2]$ | $[CaCl_2]$ | $[MgCl_2]$ | $\{Zn^{2+}\}$ | $\{Ca^{2+}\}$ | $\{Mg^{2+}\}$ | $\{Zn^{2+}\}_0$ | $RRL_{obs}$ | $RRL_{comp}$ |
|---|---|---|---|---|---|---|---|---|
| 0 | 2 | 0 | 0 | 1.91 | 0 | 0 | 100 | 100 |
| 0.02 | 1 | 0 | 0.0153 | 0.782 | 0 | 0.268 | 36 | 45 |
| 0.02 | 3 | 0 | 0.0131 | 2.00 | 0 | 0.0834 | 67 | 73 |
| 0.02 | 5 | 0 | 0.0118 | 3.04 | 0 | 0.0480 | 81 | 83 |
| 0.06 | 2 | 0.01 | 0.0418 | 1.42 | 0.00711 | 0.377 | 32 | 39 |
| 0.06 | 2 | 0.02 | 0.0418 | 1.42 | 0.0142 | 0.375 | 54 | 55 |

*Table 2.* Summary of statistics from regression analyses according to the equation $RRL = 100\left(1/\exp\left[(a_{11}/(1+a_{12}\{Ca^{2+}\}_0+a_{13}\{Mg^{2+}\}_0)\{T\}_0)^{b1}\right]\right)\left(1-1/\exp\left[(a_2\{Ca^{2+}\}_0)^{b2}\right]\right)$. $\{T\}_0$ refers to one of the toxicants below. $\{T\}_0$, $\{Ca^{2+}\}_0$, and $\{Mg^{2+}\}_0$ are expressed in mM. All presented values are significantly different from zero at the 5% level. The term $(1 - 1/\exp[(a_2\{Ca^{2+}\}_0)^{b2}])$ was omitted from the equation in the case of $Cu^{2+}$ and $Zn^{2+}$ because $\{Ca^{2+}\}_0$ was high enough for the term to equal 1. Blank spaces under $a_{12}$ and $a_{13}$ indicate values not significantly different from zero.

| Toxicant | $R^2$ | $n$ | $a_{11}$ | $a_{12}$ | $a_{13}$ | $b_1$ | $a_2$ | $b_2$ |
|---|---|---|---|---|---|---|---|---|
| $Na^+$ | 0.886 | 105 | 0.0107 | 2.04 | 0.247 | 3.51 | 12.4 | 0.555 |
| $H^+$ | 0.893 | 117 | 7.57 | 0.0863 | | 6.23 | 13.7 | 0.379 |
| $Al^{3+}$ | 0.944 | 57 | 120 | | | 2.71 | 10.3 | 0.520 |
| $Cu^{2+}$ | 0.805 | 39 | 286 | | | 0.656 | | |
| $Zn^{2+}$ | 0.938 | 66 | 3.95 | | 10.7 | 1.19 | | |

$Mg^{2+}$ alleviates toxicity in the $10^{-5}$ M range warrants dedicated study.

Table 2 provides information regarding Mechanism II. Apparently, the coefficients for Equation 2 are generally $a_2 = 12$ mM$^{-1}$ and $b_2 = 0.5$, and toxicants do not appear to affect their values. The absolute requirement for cell-surface $Ca^{2+}$ (which cannot be met with any other ion) fulfills a function with which toxicants (at least $Al^{3+}$, $Na^+$, and $H^+$) do not interfere, but the data and analyses are incomplete on that score.

Table 2 provides no information regarding Mechanism I, but Figure 5 illustrates how Mechanism I alleviation occurs, even though it is a minor factor in some cases. The solid lines indicate the decline in surface activities of the toxicants upon the addition of $CaCl_2$ or $MgCl_2$. Responsiveness is determined by two factors. First, the higher the valence, the higher the responsiveness of the toxicant to changes in $\psi_0$, as described by the Nernst equation in which charge has an exponential effect. Second, the more negative the potential initially, the greater the response of $\psi_0$ to the addition of salts. Note that $\psi_0$ at 50% inhibition is negative in the order $Cu^{2+} > Zn^{2+} > Al^{3+} > H^+ > Na^+$. Surface negativity is greatly reduced in $Na^+$ intoxicated cells despite the single charge and weak binding of $Na^+$. It is the high concentration of $Na^+$ that causes the depolarization.

There appears to be a discrepancy between Figures 4 and 5. Toxicities induced by $Na^+$ and $H^+$ are alleviated strongly by small additions of $CaCl_2$ (Figure 4), but $\{Na^+\}_0$ and $\{H^+\}_0$ are reduced weakly. This discrepancy is explained by the Mechanism III alleviation from $Ca^{2+}$ (coefficient $a_{12}$) and by the steepness of the growth response to $\{T\}_0$ in the vicinity of 50% inhibition as controlled by coefficient $b_1$ and illustrated in Figure 2. The opposite trend for $Cu^{2+}$ and $Zn^{2+}$ is explained by a lack of Mechanism III alleviation induced by $Ca^{2+}$ and by lower values for $b_1$, making responses to $\{T\}_0$ weak in the vicinity of 50% inhibition.

These and previous studies demonstrate that appropriate nonlinear equations incorporating cell-surface ion activities enable the dissection of multiple toxic and ameliorative effects of the ions.

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